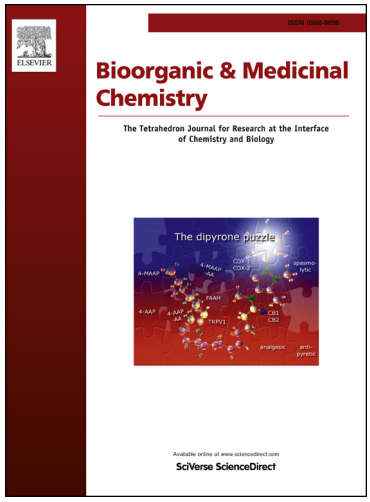

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Structure–activity relationship study of 4-(thiazol-5-yl)benzoic acid derivatives as potent protein kinase CK2 inhibitors

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ABSTRACT

Two classes of modified analogs of 4-(thiazol-5-yl)benzoic acid-type CK2 inhibitors were designed. The azabenzene analogs, pyridine- and pyridazine-carboxylic acid derivatives, showed potent protein kinase CK2 inhibitory activities [IC_{50} (CK2α) = 0.014–0.017 μM; IC_{50} (CK2α') = 0.0046–0.010 μM]. Introduction of a 2-halo- or 2-methoxy-benzyloxy group at the 3-position of the benzoic acid moiety maintained the potent CK2 inhibitory activities [IC_{50} (CK2α) = 0.014–0.016 μM; IC_{50} (CK2α') = 0.0088–0.014 μM] and led to antiproliferative activities [CC_{50} (A549) = 1.5–3.3 μM] three to six times higher than those of the parent compound.

1. Introduction

Protein kinase CK2 (previously known as casein kinase 2) is a ubiquitous, highly pleiotropic serine/threonine-specific protein kinase.[1,2] It is often present as a heterotetramer composed of two catalytic α subunits (α or α') and two regulatory β subunits in various combinations.[3,4] The CK2α' subtype is exclusively found in brain and testis, whereas the CK2α subtype is ubiquitously expressed.[5] CK2 is a key regulator of various cellular events, including signal transduction, transcriptional control, apoptosis, and cell cycle.[6–8] More importantly, overexpression and elevated activity of CK2 are closely related to many human diseases,[9–13] including cancers of the breast,[9] lung,[10] and pancreas,[11] leukemia,[12] and glomerulonephritis.[13] CK2 is therefore an important pharmacological target.[14–16]

Inhibition of CK2 decreases cellular proliferation and induces apoptosis in cancer cells. Several types of CK2 inhibitors, including natural products, have been reported, such as emodine,[17] 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBB),[18] (5-oxo-5,6-dihydroindolo[1,2-*a*]quinazolin-7-yl)acetic acid (IQA),[19] CX-4945 (orally bioactive inhibitor, currently undergoing clinical trials for cancer treatment),[20,21] and CC-4791[22,23] (Fig. 1). Recently, our group identified CK2 inhibitors with a phenylazole scaffold through virtual screening of a compound database, based on the crystal structure of the CK2α–AMP-PNP complex.[24] Structural optimization of the azole and amide moieties led to identification of amido-substituted thiazolyl- and pyrazolyl-benzoic acid derivatives **1** and **2**.[25] We then investigated the introduction of ring fusion for conformational restriction, to obtain the planar horseshoe-shaped conformation required for binding of CC-4791 to CK2α[26,27] and to reduce entropic loss during binding. Benzindazole derivative **3**[28] and indolopyrazole **4**,[29] derived from the pyrazole-based inhibitor **2**, were identified as potent CK2 inhibitors. Further structural optimization was achieved by modification of the benzoic acid moiety. In this study, the effects of replacement of benzoic acid with a pyridine- or pyridazine-carboxylic acid and introduction of hydrophobic substituents were investigated to obtain further structure–activity relationship information.

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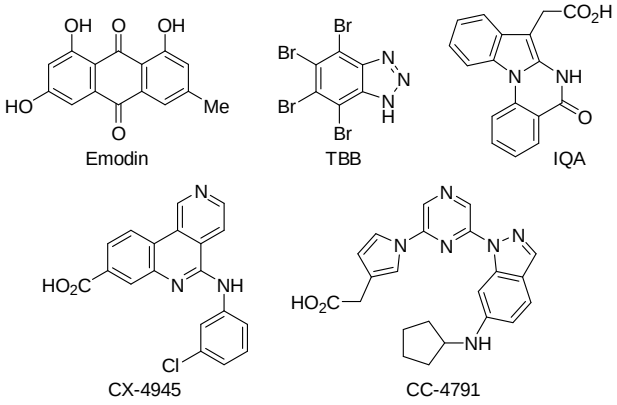


Figure 1. Representative CK2 inhibitors.

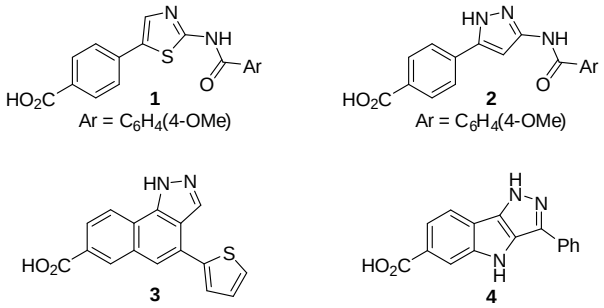


Figure 2. CK2 inhibitors developed by our group.

2. Results and discussion

2.1. Design

We designed the aza analogs **5a**–**c** (Fig. 3), based on binding affinity predictions, of CK2α–inhibitor complexes by the thermodynamic integration (TI) method using Amber.[30] The calculations suggested that replacement of the carbon atom at the 2- or 3-position of the benzoic acid moiety of **1** with a nitrogen atom (X or Y = N: **5a** and **5b**) would increase the affinities five- to ten-fold. Replacement of both carbon atoms with nitrogen atoms (X = Y = N: **5c**) might further improve the binding affinity. We also expected that the aqueous solubilities of the aza analogs would be better than that of the parent benzoic acid **1** because of additional hydrophilic interactions of the pyridine-type nitrogen(s). Our findings that nicotinic acid is a potential CK2 binder support this design concept.[31]

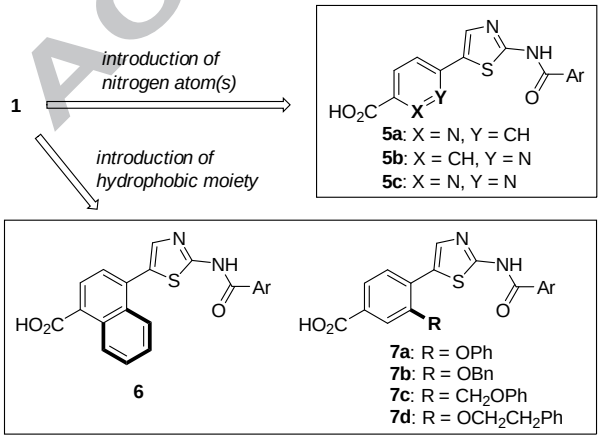


Figure 3. Design of CK2 inhibitors **5**, **6**, and **7**.

Many CK2 inhibitors have common substructures, including sp^2-hybridized heteroatom(s) (C=N or C=O) and acidic functional group(s) (CO_2H, ArOH, or triazole) (Figs. 1 and 2). Our previous studies showed that these motifs in CC-4791 and the compound **1** are important for the formation of hydrogen bonds with the backbone amide group (Val116) or electrostatic interaction with Lys68 of CK2 (Fig. 4).[25–27] Our inhibitors **1**–**4** lack the hydrophobic group present in CX-4945 (chlorophenyl group) and CC-4791 (indazole ring bearing a cyclopentylamino group). Binding mode analyses of CX-4945 and CC-4791 suggested that these groups occupy the hydrophobic pocket of CK2; this helps to improve the binding efficacy and decrease the desolvation energy. Based on these findings, we examined the introduction of ring fusion or a hydrophobic group into the benzoic acid moiety. Naphthoic acid derivative **6** and 3-substituted benzoic acid derivatives **7a**–**d** (R = OPh, OBn, CH_2OPh, or OCH_2CH_2Ph) were designed (Fig. 3).

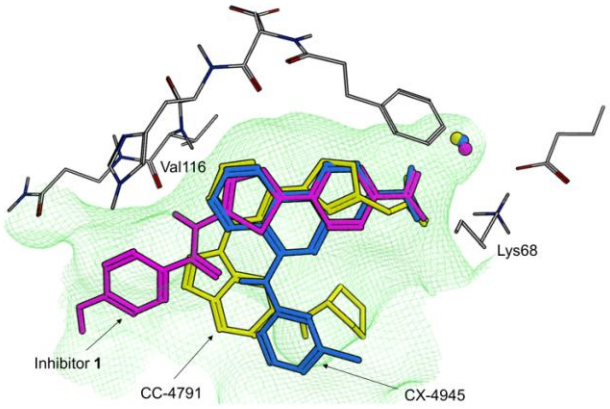
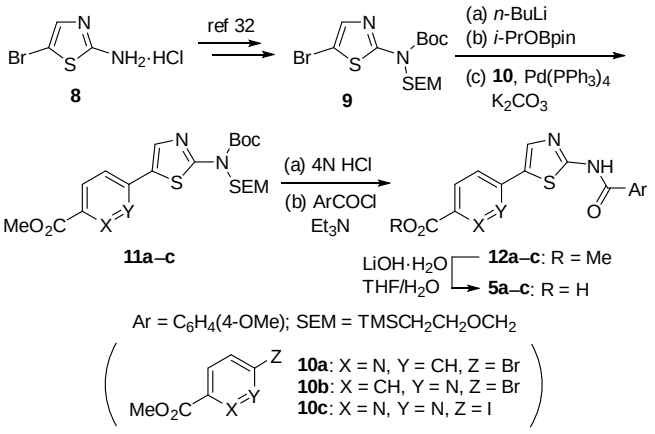


Figure 4. Superposition of CX-4945 (blue), CC-4791 (yellow), and inhibitor **1** (magenta) at ATP binding site. PDBIDs for CK2 complexes with CX-4945, CC-4791, and inhibitor **1** are 3PE1, 3AT3, and 5B0X, respectively.

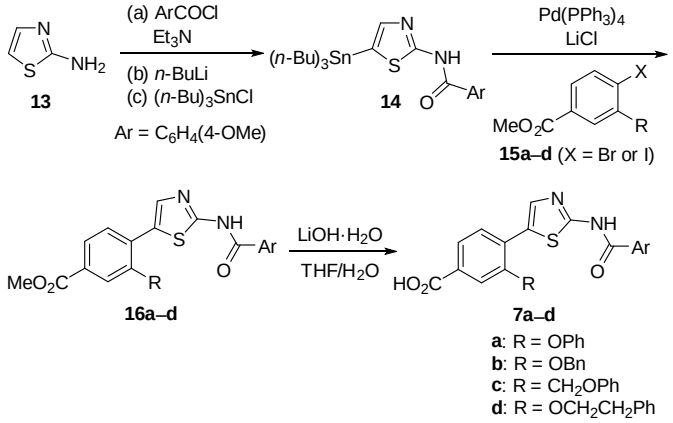
2.2. Synthesis

The aza analogs **5a**–**c** were prepared from 5-bromothiazol-2-amine hydrochloride **8** (Scheme 1). A reported procedure[32] was used to protect the amino group of **8** with Boc and 2-(trimethylsilyl)ethoxymethyl (SEM) groups. Conversion of **9** to the corresponding pinacol (pin) borane by treatment with *n*-BuLi and *i*-PrOBpin, followed by Suzuki–Miyaura cross coupling with aryl halides **10a**–**c** bearing a methoxycarbonyl group at the 4-position, afforded the coupling products **11a**–**c**. Removal of the Boc and SEM groups, acylation, and hydrolysis gave the desired aza analogs **5a**–**c**.



Scheme 1. Synthesis of aza analogs **5a**–**c**.

The synthesis of 3-substituted benzoic acid derivatives **7a**–**d** is shown in Scheme 2. Commercially available thiazol-2-amine **13** was converted to stannane **14** through acylation of the amino group and regioselective lithiation–stannylation at the 5-position. Migita–Kosugi–Stille cross coupling of **14** with halides **15a**–**d** followed by hydrolysis of the ester gave the substituted benzoic acid derivatives **7a**–**d**. Other substituted derivatives, i.e., **6** and **17a**–**h**, were prepared in a similar manner (see supplementary data).

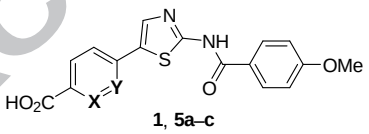


Scheme 2. Synthesis of benzoic acid derivatives **7a**–**d**.

2.3. CK2 inhibitory and cell antiproliferative activities

The in vitro inhibitory activities of the aza analogs **5a**–**c** toward two subtypes of the catalytic subunit of CK2 are shown in Table 1. As predicted by the TI calculations, **5a**–**c** showed highly potent inhibitory activities toward CK2α (IC_{50} = 0.014–0.017 μM) and CK2α' (0.0046–0.010 μM). These are comparable to the activities of CX-4945 and the parent compound **1**, but significant improvements were not observed. However, **5a**–**c** did not inhibit proliferation of lung cancer cells A549 at 30 μM. This is partly because the basic pyridine or pyridazine moiety decreases the membrane permeabilities of **5a**–**c**.[33]

Table 1
CK2 inhibitory and cell antiproliferative activities of pyridine- and pyridazine-carboxylic acid derivatives **5a**–**c**

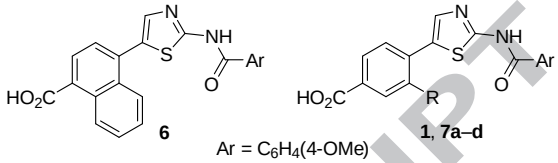


1, **5a**–**c**

Compound	X	Y	IC_{50} (μM)[a]		CC_{50} (μM)[b]
			CK2α	CK2α'	
CX-4945	–	–	0.019	0.011	9.9
1	CH	CH	0.020	0.011	8.5
5a	N	CH	0.017	0.0046	>30
5b	CH	N	0.014	0.010	>30
5c	N	N	0.014	0.0096	>30

[a] IC_{50} values were derived from the dose–response curves generated from duplicate data points of the CK2 kinase assay. [b] CC_{50} values were determined by the MTS assay against the A549 lung cancer cells after 72 h exposure to the compound.

Table 2
CK2 inhibitory and cell antiproliferative activities of benzoic acid derivatives **6** and **7a**–**d**



6 **1**, **7a**–**d**
Ar = C_6H_4(4-OMe)

Compound	R	IC_{50} (μM)[a]		CC_{50} (μM)[b]
		CK2α	CK2α'	
CX-4945	–	0.019	0.011	9.9
1	H	0.020	0.011	8.5
6	–	1.3	0.28	>30
7a	OPh	0.015	0.010	4.7
7b	OBn	0.019	0.012	1.5
7c	CH_2OPh	0.028	0.012	21.9
7d	OCH_2CH_2Ph	0.015	0.011	3.6

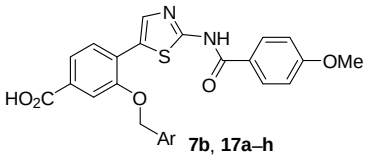
[a] IC_{50} values were derived from the dose–response curves generated from duplicate data points of the CK2 kinase assay. [b] CC_{50} values were determined by the MTS assay against the A549 lung cancer cells after 72 h exposure to the compound.

We next focused on benzoic acid derivatives bearing an additional hydrophobic moiety (Table 2). Replacement of the benzene ring of **1** by a naphthalene ring to give **6** significantly decreased the inhibitory activity toward both CK2α (IC_{50} = 1.3 μM) and CK2α' (0.28 μM). In contrast, introduction of an alkoxy group retained the potent inhibitory activities toward CK2: the phenoxy (**7a**), benzyloxy (**7b**), phenoxymethyl (**7c**), and 2-phenylethoxy (**7d**) derivatives were highly potent toward CK2α (IC_{50} = 0.015–0.028 μM) and CK2α' (0.010–0.012 μM). Promising cell antiproliferative activities against A549 were observed with **7a** (CC_{50} = 4.7 μM), **7b** (1.5 μM), and **7d** (3.6 μM). Encouraged by these results, we further optimized the structure of the 3-substituted benzoic acid derivatives. The 3-benzyloxy derivative **7b** was selected as the lead compound for further optimization, because of its higher cell antiproliferative activity and ease of derivatization because of the wide availability of substituted benzyl halides.

A series of 3-benzyloxy-substituted analogs **17a**–**h** were prepared by Migita–Kosugi–Stille cross coupling using a variety of substituted 4-iodobenzoate derivatives of type **15** (see supplementary data). The biological activities of **17a**–**h** are summarized in Table 3. Among the chlorinated benzyl ethers **17a**–**c**, the inhibitory activities of the 2-chloro derivative **17a** toward CK2α (IC_{50} = 0.016 μM) and CK2α' (0.0098 μM) were higher than those of **7b**. The inhibitory activities of 4-chloro derivative **17c** toward CK2α (IC_{50} = 0.026 μM) and CK2α' (0.019 μM) were lower than those of **17a**. For a reason that is unclear, 3-chloro derivative **17b** showed significantly lower CK2 inhibitory activities [IC_{50} (CK2α) = 0.33 μM; IC_{50} (CK2α') = 0.16 μM] and cell antiproliferative activity (CC_{50} = 18.7 μM). Highly potent inhibitory activities against CK2 were observed with 2-

halophenyl derivatives **17d** (fluorine) and **17e** (bromine). Other 2-substituted derivatives, including 2-methoxyphenyl (**17f**), 2-cyanophenyl (**17g**), and 2-pyridyl (**17h**) derivatives showed potent CK2 inhibitory activities [IC_{50} (CK2α) = 0.014–0.016 μM; IC_{50} (CK2α') = 0.0095–0.013 μM], although no antiproliferative activities were detected with **17g** and **17h** at 30 μM. All the 2-halo derivatives and the 2-methoxy-substituted one showed good antiproliferative activities against A549 (CC_{50} = 1.5–3.3 μM). Docking simulation of the potent inhibitors **17a**, **17e**, and **17f** suggested that these derivatives bind to CK2 at ATP binding site in a similar manner to CX-4945, CC-4791, and the inhibitor **1** (Figure S1, supplementary data). As we expected, the substituted phenyl group of **17** newly introduced in this study occupies the hydrophobic pocket of CK2.

Table 3
CK2 inhibitory and cell antiproliferative activities of benzyloxy-substituted derivatives **17a**–**h**



7b, **17a–h**

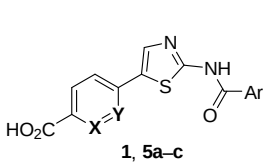
Compound	Ar	IC_{50} (μM)[a]		CC_{50} (μM)[b]
		CK2α	CK2α'	
CX-4945	–	0.019	0.011	9.9
1	–	0.020	0.011	8.5
7b	C_6H_5	0.019	0.012	1.5
17a	C_6H_4(2-Cl)	0.016	0.0098	1.6
17b	C_6H_4(3-Cl)	0.33	0.16	18.7
17c	C_6H_4(4-Cl)	0.026	0.019	2.5
17d	C_6H_4(2-F)	0.014	0.0088	3.3
17e	C_6H_4(2-Br)	0.017	0.014	1.5
17f	C_6H_4(2-OMe)	0.014	0.013	2.0
17g	C_6H_4(2-CN)	0.016	0.011	>30
17h	2-pyridyl	0.015	0.0095	>30

[a] IC_{50} values were derived from the dose–response curves generated from duplicate data points of the CK2 kinase assay. [b] CC_{50} values were determined by the MTS assay against the A549 cells after 72 h exposure to the compound.

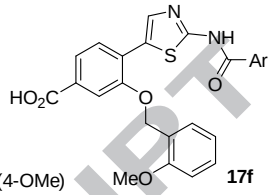
2.4. Aqueous solubility

The aqueous solubilities of representative inhibitors were evaluated (Table 4). The 2-aza (**5a**) and 3-aza analogs (**5b**) are five to 33 times more soluble (14.3–89.9 μg/mL) in phosphate buffer at pH 7.4 than **1** (2.7 μg/mL). The introduction of two nitrogen atoms (**5c**) significantly improves the solubility (1.025 mg/mL). In contrast, the introduction of 2-methoxybenzyl ether (**17f**) does not have a positive effect on the aqueous solubility (1.4 μg/mL). As described above, the aza analogs **5a**–**c** showed no antiproliferative activities at 30 μM. The 2-halo- (**17a**, **17d**, and **17e**) and 2-methoxy- (**17f**) benzyl ether derivatives, which have antiproliferative activities three to six times more potent than that of the parent compound **1**, are promising potent CK2 inhibitors, although the solubility problem needs to be solved.

Table 4
Aqueous solubilities and biological activities of **5a**–**c** and **17f**



1, **5a–c**



17f

Ar = C_6H_4(4-OMe)

Compound	X	Y	Solubility in buffer (μg/mL)[a]	IC_{50} (μM)[b]	CC_{50} (μM)[c]
CX-4945	–	–	57	0.019	9.9
1	CH	CH	2.7	0.020	8.5
5a	N	CH	14	0.017	>30
5b	CH	N	89	0.014	>30
5c	N	N	1025	0.014	>30
17f	–	–	1.4	0.014	2.0

[a] Solubility in 67 mM phosphate buffer (pH 7.4). [b] IC_{50} values were derived from the dose–response curves generated from duplicate data points of the CK2α kinase assay. [c] CC_{50} values were determined by the MTS assay against the A549 cells after 72 h exposure to the compound.

3. Conclusions

Modification of the benzoic acid moiety of 4-(thiazol-5-yl)benzoic acid-type CK2 inhibitor was investigated. As the TI calculations predicted, replacement of the benzoic acid with pyridine- or pyridazine-carboxylic acid maintained the highly potent inhibitory activities toward CK2. However, no antiproliferative activities at 30 μM were observed with these aza analogs. In contrast, the introduction of 2-halo- and 2-methoxy-substituted benzyloxy groups into the benzoic acid moiety was highly effective in improving the antiproliferative activity, leading to the identification of promising CK2 inhibitor candidates.

4. Experimental

General Methods. ^1H NMR spectra were recorded using a JEOL AL-500 spectrometer at 500 MHz frequency. Chemical shifts are reported in δ (ppm) relative to Me_4Si (in $CDCl_3$) as internal standard. ^{13}C NMR spectra were recorded using a JEOL AL-500 and referenced to the residual $CHCl_3$ signal. IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer or Shimadzu LC-ESI-IT-TOF-MS equipment (ESI). Melting points were measured by a hot stage melting points apparatus (uncorrected). For column chromatography, Wakogel C-300E (Wako), Chromatorex NH-DM1020 (Fuji Silysia), or Aluminum oxide 90 (Merck-Millipore) was employed. Several benzoic acid derivatives (**7a**, **17b** and **17g**) were further purified by HPLC [Cosmosil 5C18-ARII column (20 × 250 mm, Nacalai Tesque, Inc.); water/acetonitrile

containing 0.1% NH_3; linear gradient; flow rate of 8 mL/min; UV detector at 254 nm] to afford their ammonia salts as lyophilized powders.

General Procedure for Suzuki-Miyaura Cross Coupling: Synthesis of Methyl 5-{2-((*tert*-Butoxycarbonyl){[2-(trimethylsilyl)ethoxy]methyl]amino)thiazol-5-yl]picolinate (11a). *n*-BuLi (1.55 M solution in hexane; 0.484 mL, 0.75 mmol) was added slowly to a mixture of bromide **9**[31] (204 mg, 0.5 mmol) in THF (5 mL) at –78 °C under argon. *i*-PrOBpin (152 μL, 0.75 mmol) was added to the mixture, and the resulting mixture was then allowed to warm to 0 °C. 2 N NH_4Cl was added to the mixture. The mixture was partitioned between EtOAc and brine, and the layers were separated. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a residual oil. A mixture of this crude borate, bromopyridine **10a**[34] (129 mg, 0.6 mmol), $Pd(PPh_3)_4$ (5.8 mg, 0.005 mmol), and K_2CO_3 (207 mg, 1.5 mmol) in DMF (5 mL) under argon was stirred at 80 °C overnight. After being cooled to room temperature, the solvent was removed under reduced pressure and the residue was diluted with CH_2Cl_2 and H_2O. The organic phase was washed with brine and dried over $MgSO_4$. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over silica gel with EtOAc/hexane to give **11a** (88.6 mg, 38%) as a white solid: mp 128–130 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 40 °C) δ: –0.02 (s, 9H, $Si(CH_3)_3$), 0.95 (t, *J* = 8.3 Hz, 2H, CH_2), 1.60 (s, 9H, $C(CH_3)_3$), 3.69 (t, *J* = 8.3 Hz, 2H, OCH_2), 4.00 (s, 3H, OCH_3), 5.57 (s, 2H, NCH_2O), 7.78 (s, 1H, Ar), 7.92 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar), 8.11 (d, *J* = 8.0 Hz, 1H, Ar), 8.88 (d, *J* = 1.7 Hz, 1H, Ar); ^{13}C NMR (125 MHz, $CDCl_3$, 40 °C) *δ*: –1.4 (3C), 18.2, 28.1 (3C), 52.9, 67.1, 75.7, 84.4, 125.4, 128.7, 131.6, 133.2, 136.1, 146.0, 146.5, 152.8, 161.8, 165.3; HRMS (FAB^+) calcd for $C_{21}H_{32}N_3O_5SSi$ $[M + H]^+$: 466.1826; found: 466.1832.

General Procedure for Deprotection and *N*-Acylation: Synthesis of Methyl 5-[2-(4-Methoxybenzamido)thiazol-5-yl]picolinate (12a). HCl (4 N in dioxane; 8 mL) was added to a solution of **11a** (233 mg, 0.5 mmol) in dioxane (2 mL), and the mixture was stirred at room temperature for 16 h. After evaporation of the solvent, a mixture of this crude product, 4-methoxybenzoyl chloride (101 μL, 0.75 mmol) and Et_3N (208 μL, 3.0 mmol) in THF (10 mL) was stirred at 0 °C overnight. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc and H_2O. The organic phase was washed with brine, and dried over $MgSO_4$. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over NH-silica gel with $CHCl_3$–MeOH to give **12a** (160 mg, 86%) as a pale yellow solid: mp 263–265 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 40 °C) δ: 3.90 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.03 (d, *J* = 8.6 Hz, 2H, Ar), 7.67 (s, 1H, Ar), 7.92–7.99 (m, 3H, Ar), 8.15 (d, *J* = 8.0 Hz, 1H, Ar), 8.92 (s, 1H, Ar), 10.77 (br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$, 40 °C) *δ*: 52.9, 55.6, 114.4 (2C), 124.0, 125.5, 128.2, 129.8 (2C), 131.4, 133.5, 135.6, 146.5, 146.6, 160.1, 163.8, 164.5, 165.3; HRMS (FAB^+) calcd for $C_{18}H_{16}N_3O_4S$ $[M + H]^+$: 370.0856; found: 370.0861.

General Procedure for Hydrolysis: Synthesis of 5-[2-(4-Methoxybenzamido)thiazol-5-yl]picolinic Acid (5a). 1 N LiOH (750 μL, 0.75 mmol) was added to a stirred mixture of **12a** (92.3 mg, 0.25 mmol) in THF (5 mL) and water (5 mL), and the mixture was stirred for 24 h at room temperature. The mixture was acidified by 1 N HCl until pH < 2, then cooled to 0 °C. The yellow precipitate was collected by filtration, washed with water, and dried under vacuum to give **5a** (71.5 mg, 81%) as a yellow solid: mp 274–276 °C; ^{1}H-NMR (500 MHz, DMSO-d_6, 60 °C) δ: 3.87 (s, 3H, OCH_3), 7.08 (d, *J* = 8.6 Hz, 2H, Ar), 8.08 (d, *J* = 7.4 Hz, 1H, Ar), 8.13 (d, *J* = 8.6 Hz, 2H, Ar), 8.19–8.21 (m, 2H, Ar), 9.01 (s, 1H, Ar); ^{13}C NMR (125 MHz, DMSO-d_6, 60 °C) *δ*: 55.3, 113.7 (2C), 123.7, 124.8, 126.5, 130.1 (2C), 130.8, 133.4, 136.6, 145.6, 146.2, 159.6, 162.7, 164.5, 165.3; HRMS (FAB^+) calcd for $C_{17}H_{14}N_3O_4S$ $[M + H]^+$: 356.0700; found: 356.0705.

CK2 Kinase Assay. CK2 inhibitory activities were evaluated by the off-chip mobility shift assay by the QuickScout® service from Carna Bioscience (Kobe, Japan). Human GST-fusion CK2α(1-391) or CK2α'(1-350) was co-expressed with His-tagged human CK2β(1-215) using baculovirus expression system. GST-CK2α and GST-CK2α' were purified by using glutathione sepharose chromatography. Each chemical in DMSO at different concentrations was diluted fourfold with reaction buffer [20 mM HEPES (pH 7.5), 0.01% Triton X-100, 2 mM DTT]. For CK2 reactions, a combination of the compound, 1 μM CK2tide, 5 mM $MgCl_2$, 5 μM ATP in reaction buffer (20 μL) were incubated with each CK2 in PP 384-well plates at room temperature for 1 h (*n* = 2). The reaction was terminated by addition of 60 μL of termination buffer (Carna Biosciences). Substrate and product were separated by electrophoretic means using the LabChip3000 system. The kinase reaction was evaluated by the product ratio, which was calculated from the peak heights of the substrate (S) and product (P): [P/(P+S)]. Inhibition data were calculated by comparing with no-enzyme controls for 100% inhibition and no-inhibitor reactions for 0% inhibition. IC_{50} values were calculated using GraphPad Prism software.

Growth Inhibition Assay. A549 cell was cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma), supplemented with 10% (v/v) fetal bovine serum at 37 °C in a 5% CO_2-incubator. Growth inhibition assays using these cells were performed in 96-well plates (BD Falcon). A549 cell was seeded at 500 cells/well in 60 μL of culture media, and were cultured for 6 h. Then, 60 μL of the chemical diluents were added, and the cells under chemical treatment were incubated for further 72 h. The final volume of DMSO in the medium was equal to 0.3% (v/v). The wells in the plates were washed twice with the cultured medium without phenol-red. After 1 h incubation with 100 μL of the medium, the cell culture in each well was supplemented with 20 μL of the MTS reagent (Promega), followed by incubation for additional 40 min. Absorbance at 490 nm of each well was measured using a EnVision Xcite Multilabel Reader (Perkin Elmer). CC_{50} values were calculated using GraphPad Prism software (*n* = 2).

Thermodynamic Solubility in Aqueous Solution. An equal volume of a mixture of 1/15 M phosphate buffer (pH 7.4, 0.5 mL) and EtOH (0.5 mL), or 1/15 M phosphate buffer (pH 7.4, 1.0 mL) was added to a compound in a vial. The suspension was then shaken for 24 h at 37 °C, and undissolved material was separated by filtration. *m*-Cresol was added as an internal standard (final concentration: 0.05 mg/mL) and the mixture was diluted in 0.1% NH_3 and injected onto the HPLC column. The peak area ratio of the sample to the standard was recorded by UV detection at 254 nm. The concentration of the sample solution was calculated using a previously determined calibration curve, corrected for the dilution factor of the sample.

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Supplementary data

Supplementary data associated with this article (provided by the authors including experimental procedures and characterization data for all new compounds) can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc...

References and notes

1. Burnett, G.; Kennedy, E. P. *J. Biol. Chem*. **1954**, *211*, 969–980.
2. Salvi, M.; Sarno, S.; Cesaro, L.; Nakamura, H.; Pinna, L. A. *Biochim. Biophys. Acta* **2009**, *1793*, 847–859.
3. Litchfield, D. W. *Biochem. J*. **2003**, *369*, 1–15.
4. Filhol, O.; Martiel, J. L.; Cochet, C. *EMBO Rep*. **2004**, *5*, 351–355.
5. Guerra, B.; Siemer, S.; Boldyreff, B.; Issinger, O.-G. *FEBS Lett*. **1999**, *462*, 353–357.
6. Filhol, O.; Cochet, C. *Cell. Mol. Life Sci*. **2009**, *66*, 1830–1839.
7. Trembley, J. H.; Wang, G.; Unger, G.; Slaton, J.; Ahmed, K. *Cell. Mol. Life Sci*. **2009**, *66*, 1858–1867.
8. Loizou, J. I.; El-Khamisy, S. F.; Zlatanou, A.; Moore, D. J.; Chan, D. W.; Qin, J.; Sarno, S.; Meggio, F.; Pinna, L. A.; Caldecott, K. W. *Cell* **2004**, *117*, 17–28.
9. Giusiano, S.; Cochet, C.; Filhol, O.; Duchemin-Pelletier, E.; Secq, V.; Bonnier, P.; Carcopino, X.; Boubli, L.; Birnbaum, D.; Garcia, S.; Iovanna, J.; Charpin, C. *Eur. J. Cancer* **2011**, *47*, 792–801.
10. Zhao, T.; Jia, H.; Li, L.; Zhang, G.; Zhao, M.; Cheng, Q.; Zheng, J.; Li, D. *Oncol. Rep*. **2013**, *30*, 377–384.
11. Giroux, V.; Dagorn, J. C.; Iovanna, J. L. *Pancreatology* **2009**, *9*, 738–754.
12. Borgo, C.; Cesaro, L.; Salizzato, V.; Ruzzene, M.; Massimino, M. L.; Pinna, L. A.; Donella-Deana, A. *Mol. Oncol*. **2013**, *7*, 1103–1115.
13. Yamada, M.; Katsuma, S.; Adachi, T.; Hirasawa, A.; Shiojima, S.; Kadowaki, T.; Okuno, Y.; Koshimizu, T.; Fujii, S.; Sekiya, Y.; Miyamoto, Y.; Tamura, M.; Yumura, W.; Nihei, H.; Kobayashi, M.; Tsujimoto, G. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 7736–7741.
14. Dobrowolska, G.; Lozeman, F. J.; Li, D.; Krebs, E. G. *Mol. Cell. Biochem*. **1999**, *191*, 3−12.
15. Guerra, B.; Issinger, O. G. *Curr. Med. Chem*. **2008**, *15*, 1870–1886.
16. Ruzzene, M.; Pinna, L. A. *Biochim. Biophys. Acta* **2010**, *1804*, 499–504.
17. Yim, H. L.; Lee, Y. H.; Lee, C. H.; Lee, S. K. *Planta Med.* **1999**, *65*, 9–13.
18. Szyszka, R.; Grankowski, N.; Felczak, K.; Shugar, D. *Biochem. Biophys. Res. Commun.* **1995**, *208*, 418–424.
19. Vangrevelinghe, E.; Zimmermann, K.; Schoepfer, J.; Portmann, R.; Fabbro, D.; Furet, P. *J. Med. Chem.* **2003**, *46*, 2656–2662.
20. Pierre, F.; Chua, P. C.; O'Brien, S. E.; Siddiqui-Jain, A.; Bourbon, P.; Haddach, M.; Michaux, J.; Nagasawa, J.; Schwaebe, M. K.; Stefan, E.; Vialettes, A.; Whitten, J. P.; Chen, T. K.; Darjania, L.; Stansfield, R.; Anderes, K.; Bliesath, J.; Drygin, D.; Ho, C.; Omori, M.; Proffitt, C.; Streiner, N.; Trent, K.; Rice, W. G.; Ryckman, D. M. *J. Med. Chem.* **2011**, *54*, 635–654.
21. Battistutta, R.; Cozza, G.; Pierre, F.; Papinutto, E.; Lolli, G.; Sarno, S.; Brien, S. E.; Siddiqui-Jain, A.; Haddach, M.; Anderes, K.; Ryckman, D. M.; Meggio, F.; Pinna, L. A. *Biochemistry* **2011**, *50*, 8478–8488.
22. Fuchi, N.; Iura, Y.; Kaneko, H.; Yamada, M.; Sekitani, Y. *Jpn. Pat.* 145786, 2007.
23. Fuchi, N.; Iura, Y.; Kaneko, H.; Nitta, A.; Suyama, K.; Ueda, H.; Yamaguchi, S.; Nishimura, K.; Fujii, S.; Sekiya, Y.; Yamada, M.; Takahashi, T. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 4358–4361.
24. Nakanishi, I.; Murata, K.; Nagata, N.; Kurono, M.; Kinoshita, T.; Yasue M.; Miyazaki, T.; Takei, Y.; Nakamura, S.; Sakurai, A.; Iwamoto, N.; Nishiwaki, K.; Nakaniwa, T.; Sekiguchi, Y.; Hirasawa, A.; Tsujimoto, G.; Kitaura, K. *Eur. J. Med. Chem.* **2015**, *96*, 396–404.
25. Hou, Z.; Nakanishi, I.; Kinoshita, T.; Yasue, M.; Suzuki, Y.; Kanemitsu, M.; Kure, T.; Ohno, H.; Murata, K.; Kitaura, K.; Hirasawa, A.; Tsujimoto, G.; Oishi, S.; Fujii, N. *J. Med. Chem*. **2012**, *55*, 2899–2903.
26. Nakaniwa, T.; Kinoshita, T.; Sekiguchi, Y.; Tada, T.; Nakanishi, I.; Kitaura, K.; Suzuki, Y.; Ohno, H.; Hirasawa, A.; Tsujimoto, G. *Acta Cryst*. **2009**, *F65*, 75–79.
27. Kinoshita, T.; Sekiguchi, Y.; Fukada, H.; Nakaniwa, T.; Tada, T.; Nakamura, S.; Kitaura, K.; Ohno, H.; Suzuki, Y.; Hirasawa, A.; Nakanishi, I.; Tsujimoto, G. *Mol. Cell. Biochem*. **2011**, *356*, 97–105.
28. Suzuki, Y.; Oishi, S.; Takei, Y.; Yasue, M.; Misu, R.; Naoe, S.; Hou, Z.; Kure, T.; Nakanishi, I.; Ohno, H.; Hirasawa, A.; Tsujimoto, G.; Fujii, N. *Org. Biomol. Chem*. **2012**, *10*, 4907–4915.
29. Hou, Z.; Oishi, S.; Suzuki, Y.; Kure, T.; Nakanishi, I.; Hirasawa, A.; Tsujimoto, G.; Ohno, H.; Fujii, N. *Org. Biomol. Chem*. **2013**, *11*, 3288–3296.
30. Pearlman, D. A.; Case, D. A.; Caldwell, J. W.; Ross, W. S.; Cheatham III, T. E.; DeBolt, S.; Ferguson, D.; Seibel, G.; Kollman, P. *Comp. Phys. Commun.* **1995**, *91*, 1–14.
31. Kinoshita, T.; Nakaniwa, T.; Sekiguchi, Y.; Sogabe,, Y.; Sakurai, A.; Nakamura, S.; Nakanishi, I. *J. Synchrotron Rad*. **2013**, *20*, 974–979.
32. Sleebs, B. E.; Street, I. P.; Bu, X.; Baell, J. B. *Synthesis* **2010**, 1091–1096.
33. The observed lower CC_{50} values in cell proliferation assay compared with the IC_{50} in the kinase assay can be partly attributed to far higher ATP concentration (1–10 mM) in cells than those in the kinase assay (5 μM). As described, the CC_{50} values of the cell-based assay is also influenced by the membrane permeabilities of the compounds. For related discussions, see ref. 20.
34. Andernach, L.; Opatz, T. *Eur. J. Org. Chem*. **2014**, 4780–4784.

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Structure–activity relationship study of 4-(thiazol-5-yl)benzoic acid derivatives as potent protein kinase CK2 inhibitors

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